THE INFLUENCE OF FEED AND FEEDING UPON THE RUMINAL GAS FORMATION IX. FORMATE DISSIMILATION AND GAS PRODUCTION IN THE RUMEN OF THE GOAT

THE INFLUENCE OF FEED AND FEEDING UPON THE RUMINAL GAS FORMATION

IX. FORMATE DISSIMILATION AND GAS PRODUCTION IN THE RUMEN OF THE GOAT

By

Tatsuro MATSUMOTO

Department of Animal Husbandry, Faculty of Agriculture, Tohoku University, Sendai, Japan

(*Received, June 30, 1961*)

Introduction

It is now established that the volatile fatty acids in the rumen liquor consist in the main of acetic, propionic and butyric, with smaller amounts of higher fatty acids. Although formic acid has been found in the rumen under certain conditions, it is not usually present. But, in vitro experiments, formic acid is produced by cellulose splitting bacteria (1). These facts suggest that formic acid may arise as an intermediate in carbohydrate fermentation in the rumen. Claren (2) and Gray *et al.* (3) found a very small amount of formic acid in the rumen liquor and Annison (4) reported an occasional trace of formic acid, but never more than one per cent of the total volatile acids. Tyznik and Allen (5) observed that formic acid appeared in the rumen one hour after acetate administration and disappeared in a short time.

Beijer (6) noted formate dissimilation and methane production when one per cent formate was added to the contents of a flask completely filled with rumen fluid. Carroll *et al.* (7) showed the production of methane from formate in their *in vitro* tracer experiment with $HC^{14}OONa$. The present study was designed to find the influence of formate administration on the ruminal gas formation.

Experiments

Experiment 1. Rate of formate dissimilation in the rumen of the goat.

A fisturated female goat, weighing 35 kg, received the diet as shown in Table 1. The proximate analysis of feeds and formic acid contents are given in Table 2. Formate solution was ingested through the rumen fistula at 10 A.M. every day.

Table 1. Dietary regime

Time	Feeds
1 P.M.	Wheat bran 150g + barley 150g + hay 500g
4 P.M.	Hay 300g

Table 2. Percentage composition of feeds used in the experiment

	Wheat bran	Barley	Hay
Moisture	13.82	15.33	9.83
Crude protein	15.52	11.62	6.12
Crude fat	4.07	1.69	2.75
Nitrogen free extract	54.23	63.44	45.44
Crude fiber	7.72	5.84	28.03
Crude ash	4.64	2.08	7.83
Formic acid	0.0005	0.0003	0.0049

Determination of formic acid in feed and rumen liquor.

Twenty grams of pulverized feed was put in a 500 *ml* Erlenmyer flask and extracted with 200 *ml* of $N/10 - H_2SO_4$ for two hrs. at 60°C. Then the extract was centrifuged and 50 *ml* of the supernatant was analysed for formic acid using the method of A. O. A. C. (8). Rumen liquor of the goat was gathered by a catheter through the fistula and centrifuged at once. Fifty milli-litre of the supernatant was analysed for formic acid.

The total amount of formic acid in the diet, calculated from Table 1 and Table 2, was 40 mg per day. The detective reaction of formic acid (9) in the rumen liquor just before the formate administration was always negative.

Determination of total volume of rumen liquor.

The technique employed was essentially the same as that described by Kandatsu *et al.* (10). Ten grams of sodium chloride was dissolved in 70 *ml* of water, and administered through the rumen fistula. The concentration of Cl in the rumen liquor was determined by Mohr's method (11). From the difference in Cl concentration between the rumen liquor collected before and two hours after the administration of sodium chloride, the total volume of rumen liquor was calculated as follows :

$$V = M/(C_2 - C_1)$$

V : total volume of rumen liquor (l)
M : the amount of Cl administered (g)
C_1 : concentration of Cl before admsnistration (g/l)
C_2 : concentration of Cl two hours after the administration of sodium chloride (g/l)

Table 3. Total volume of rumen liquor

No. of trial	C_1	C_2	V
1	0.5816	1.2659	8.82
2	0.4961	1.1290	9.53
3	0.3803	1.0606	8.87
4	0.3344	0.8895	10.87
5	0.2595	0.9580	8.64

Mean value and standard deviation 9.35 ± 0.91 (*l*).

Different amounts of sodium formate were dissolved in 50 *ml* water and injected into the rumen of the goat at 10 A.M. and the concentrations of formic acid in the rumen liquor following the formate administration were calculated. Two hours after administration, the concentrations of formic acid in the rumen liquor were chemically determined. The results are shown in Table 4.

Table 4. Concentrations of formic acid in the rumen liquor following formate administration

H·COONa administered (g)	Concentrations of H·COOH in the rumen liquor	
	just after administration* (mg/*dl*)	2 hrs. later** (mg/*dl*)
6	43.4	0.0
10	72.4	0.0
20	144.8	0.0
30	217.2	0.8
40	289.6	18.2
50	362.0	31.2

* calculated ; ** measured.

As shown in Table 4, formic acid entirely disappeared in the cases of under 20 g, and a small amount of formic acid remained in the rumen liquor in the cases of when more than 30 g formate were administered.

The rate of disappearance of formic acid in the rumen were examined. The results are shown in Fig. 1.

The order of the reaction was calculated (13), and it was proved that the dissimilation reaction of formic acid in the rumen was of zero order.

That is, the rate of disappearance is constant over a period of time and never influenced by the initial concentration of the formic acid :

dy / dx = constant

x : time after administration

y : amount of formic acid disappeared

The rate of disappearance of formic acid in the rumen is shown in Fig. 2. Carroll *et al.* (7) used large Warburg vessels, containing 10 g of rumen content and 40 mg of sodium formate and 30 *ml* of inorganic salt solution with an

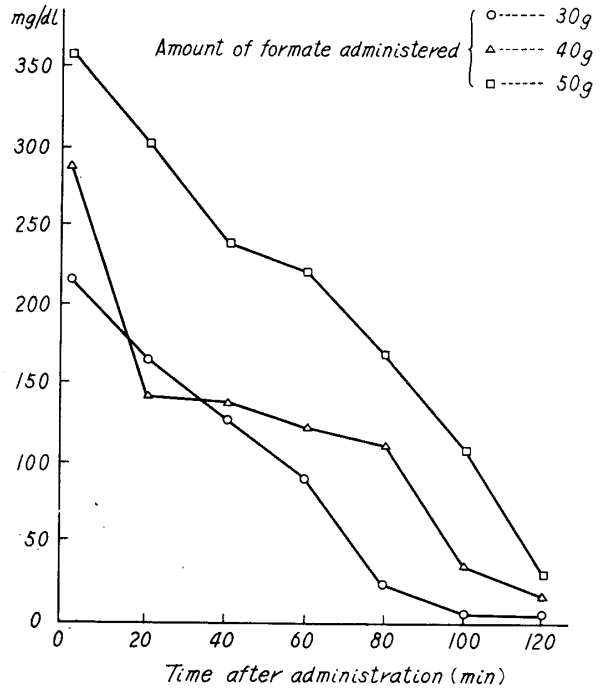

Fig. 1. Formic acid concentrations of the rumen lrquor after sodium formate admistration.

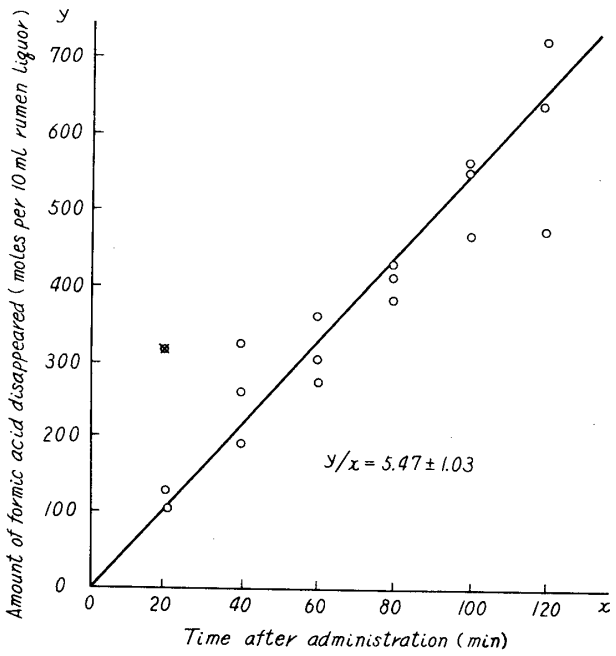

Fig. 2. The rate of disappearance of formic acid in the rumen.
⊠.....Elimininated, according to Thompson's rejecting test.

atmosphere of CO_2 and kept at 39.1° C. In their experiment, the rate of disappearance of formic acid was 6.28 ± 0.33 μ moles per 10 g rumen content per minute and in my experiment, the rate was 5.47 ± 1.03 μ moles per 10 *ml* of rumen liquor per minute. Comparing these results, it may be concluded that the disappearance of formic acid in the rumen is caused by decomposition of formic acid and not ascribable to absorption from rumen epithelium.

Experiment 2. The influence of sodium formate administration on the rumen gas compositions.

An intact female goat, weighing 32 kg, was used in Exp. 2. Feeding conditions of Exp. 2 were exactly the same as Exp. 1. The first week was the preliminary feeding period and in the second week rumen gases were collected at 10 A.M. every day. In the third week, 30 g of sodium formate was dissolved in 100 *ml* of water and injected directly into the rumen at 10 A.M. every day, and rumen gases were collected at 10 : 40 A.M.. The technique of gas collection and the method of gas analysis were described in a previous paper (12). The results are presented in Table 5.

Table 5. The influence of sodium formate administration on the rumen gas compositions

Time / Rumen gas	Just before administration	40 min after administration	Significance
CO_2 %	31.6 ±2.9	28.9 ±4.9	−
CH_4 %	40.7 ±3.8	51.8 ±6.7	+
H_2 %	1.8 ±0.4	1.2 ±1.5	−
O_2 %	4.6 ±1.7	3.7 ±0.9	−
N_2 %	21.3 ±3.3	14.3 ±5.1	−
CO_2/CH_4	0.78±0.09	0.57 ±0.14	+

+ : Difference means significant at 5 per cent level.

Methane content of the rumen gas before formate administration was 41 per cent and that of after administration was 52 per cent. The difference was statistically significant ($P<0.05$). The mean value of CO_2/CH_4 ratio decreased from 0.78 to 0.57 as a result of formate administration. This change was also statistically significant ($P<0.05$). Diminution of N_2 content after formate administration suggested the increase of ruminal gas production.

To speculate on the states of ruminal gas production, CO_2/N_2, CH_4/N_2 and H_2/N_2 ratios were calculated and are summarized in Table 6.

CO_2/N_2 ratio increased from 1.52 to 2.23, indicating an increase of CO_2 production, but, the difference was statistically insignificant. CH_4/N_2 ratio increased from 1.95 to 4.08 and the difference was statistically significant ($P<0.10$). The production of methane after formate administration was

Table 6. Comparison of rumen gas ratios before and after formate administration

Gas ratio \ Time	Just before administration	40 min after administration	Significance
CO_2 / N_2	1.52 ±0.36	2.23 ±0.86	−
CH_4 / N_2	1.95 ±0.43	4.08 ±1.54	+
H_2 / N_2	0.09 ±0.03	0.09 ±0.06	−

+ : Difference means significant at 10 per cent level.

remarkable. Both of the H_2/N_2 ratios, before and after formate administration, were 0.09, indicating that formate had no effecto n hydrogen production.

Discussion

The results of the two sets of experiments, determination of the rate of dissimilation in the rumen fluid and rumen gas analysis after formate administration, may be taken to indicate that formic acid is rapidly decomposed in the rumen and produces methane gas. Methane formation has been found to occur both with and without the reduction of carbon dioxide. In respect to menthane fermentation, Buswell *et al.* (14) set up the following empirical equation :

$$C_nH_aO_b + (n-a/4-b/2)\ H_2O \rightarrow (n/2+a/8-b/4)\ CH_4 + (n/2-a/8+b/4)\ CO_2 \quad \cdots\cdots (I)$$

In the case of formic acid, $n=1$, $a=2$, $b=2$, and the following equation may be applicable.

$$4HCOOH = CH_4 + 3CO_2 + 2H_2O \quad \cdots\cdots (II)$$

Carroll *et al.* (7) confirmed this reaction in their *in vitro* experiments and suggested that the same reaction took place in the rumen. If this conception is valid, then the amount of carbon dioxide, produced from formic acid, should be three times as much as methane. But, the results obtained in the Exp. 2 showed the decrease of CO_2/CH_4 ratio after formate administration, indicating that methane production was larger than carbon dioxide production. This may be attributed to that sodium formate was used instead of formic acid. Beijer (6) used sodium formate in his *in vitro* experiment and found that the amount of methane produced was about seven times larger than that of carbon dioxide. The results of the present study were similar to those obtained in Beijer's experiment in respect to methane and carbon dioxide production. Hydrogen was produced as much as carbon dioxide in Beijer's experiment and no increment of hydrogen production was observed in my experiment. This is a remarkable disagreement between Beijer's experiment and the present study. Carroll *et al.* (7) described the conversion process of

formate to mehane in two steps :

$$H\cdot COOH \rightarrow CO_2 + H_2 \quad \text{(III)}$$
$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad \text{(IV)}$$

Usually, the reaction (IV) must proceed faster than the reaction (III) in the rumen, so hydrogen is rarely detected in the normally fed animal. According to Pilgrim (15), hydrogen can be detected in significant amount under some conditions such as starvation. Carroll *et al.* (7) confirmed the reaction (IV) in their *in vitro* experiment. The fact that hydrogen production was observed in Beijer's experiment suggested that the velocity of reaction (IV) in a vessel was different from that of in the rumen.

In addition to formic acid, the following substances are known as substrates for the methane production (16) : acetic acid, propionic acid, butyric acid, valeric acid, methanol, ethanol, propanol, butanol, acetone, oxalic acid, succinic acid, etc.. Among these substances, acetic-, propionic- and butyric acid are always present abundantly in the rumen liquor. The experiments described above indicate that the dissimilation of formic acid takes precedence over all other fatty acids.

So far as I investigated, formic acid contents of feeds were relatively small, and thare is no reason to suppose that methane in the rumen originates from the formic acid in feeds. Formic acid is produced in so many cases of anaerobic fermentation that it may be produced as an intermediate metabolite of ruminal fermentation and decomposed to methane immediately. McNeil and Jacobson (17) have concluded that the reduction of carbon dioxide provides the main pathway for methane production in the rumen and that the availability of hydrogen is the chief limiting factor in such a reaction.

Summary

1. Formic acid was not detected in the rumen liquor of a goat fed on the mixture of hay, barley and wheat bran.
2. The rate of formic acid disappearance in the rumen was determined by the administration of sodium formate to a fistulated goat. The result obtained in the present paper was nearly the same as obtained in the *in vitro* experiment of Carroll and Hungate (7).
3. The influence of sodium formate administration on the ruminal gas production was examined. A remarkable increase of methane content and decrease of CO_2/CH_4 ratio in the rumen gas were observed.

References

1) Hungate, R.E. (1950). Bacteriol. Rev., **14**, 1.
2) Claren, O.B. (1942). Z. physiol. Chem., **276**, 97.

3) Gray, F.V., A.F. Pilgrim, H.J. Rodda and R.A. Weller (1952). J. Exp. Biol., **29**, 57.
4) Annison, E.F. (1954). Biochem. J., **57**, 400.
5) Tyznik, W. and N.N. Allen (1951). J. Dairy Sci., **34**, 493.
6) Beijer, W.E. (1952). Nature, **170**, 576.
7) Carroll, E.J. and R.E. Hungate (1955). Arch. Biochem. Biophys., **56**, 525.
8) Official Method of Analysis of the Association of Official Agricultural Chemists, 7th ed. (1950). 459.
9) Feigl, F. (1947). Qualitative Analysis by Spot Tests. Inorganic and Organic Applications (Elsevier Publishing Co. Inc. New York, Amsterdam, London, Brussels) p. 395.
10) Kandatsu, M.,N. Yazu and A. Moriyama (1956). Jap. J. Zootech. Sci., **27**, 77 (in Japanese with English summary).
11) Day, R.A. Jr. and A.L. Underwood (1959). Quantitative Analysis (Pretice Hall Inc. and Maruzen Co. Ltd.) p. 178 (Maruzen Asian Ed.).
12) Matsumoto, T. (1957). J. Agr. Chem. Soc. Japan (Nippon Nogeikagaku Kaishi) **31**, 300 (in Japanese).
13) Sakaguchi, K. and T. Uemura (1944). Chemistry of catalyser—Enzymes (Shukyo Shoin) p. 57 (in Japanese).
14) Buswell, A.M. and H.F. Mueller (1952). Ind. Eng. Chem., **44**, 550.
15) Pilgrim, A.F. (1948). Aust. J. Sci. Res., **1**, 130.
16) Barker, H.A. (1956). Ind. Eng. Chem., **48**, 1438.
17) McNeil, J.J. and D.R. Jacobson (1955). J. Dairy Sci., **38**, 608.